



ELECTRORETINOGRAPHY (A REVIEW)

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ABSTRACT

Electroretinography (ERG) is a functional test of the outer retina. During an examination, the retina is selectively stimulated. The stimulation of the retina produces a response of the individual retinal cells and reveals information about its function. The ERG examination requires very specific conditions in order to avoid undesirable factors which may adversely affect the recordings. The electroretinography examination may be performed for a short period (“rapid protocol”), commonly used to access retinal activity. The “long protocol” is used for the differential diagnosis of retinal disorders. It is mainly used in diagnosing and evaluating retinal dysfunction when there are no ophthalmic lesions present. The main indications for electroretinography are the pre-operative examination of cataract patient and the early diagnosis of inherited retinal diseases. In veterinary ophthalmology, ERG is performed under general anesthesia. The ERG results have wave forms with characteristic components depending upon several factors. Its interpretation requires knowledge of retinal pathology and electrophysiology.

Key words: electroretinography (ERG); ophthalmology; retina; retinopathy

INTRODUCTION

Electroretinography (ERG) is an electrodiagnostic technique assessing retinal function in dogs and many other animals. The first ERG examination in veterinary medicine was performed by Perry, Thomson and Tansley in 1953 [25], although the history of ERG begins 100 years earlier when DuBois-Reymond discovered the resting potential between the anterior and posterior pole of the non-stimulated eye [4]. In 1865, Holmgren measured the electrical potential over the eye as a response to the illumination of the retina which was considered as the beginning of electroretinography [9]. The first ERG examination on an animal took place in 1873 [3]. In 1953 a dog was examined for the first time [25]; feline ERG was 10 years later [29].

ERG is a non-invasive examination that provides objective information about the function of individual retinal cells [16]. There are dual protocols used in veterinary

ophthalmology. The rapid protocol (Yes/No protocol) gives information about the function of the retina. The rapid protocol is commonly used as a pre-surgical evaluation of the retina in patients being prepared for cataract surgery (before the phacoemulsification full protocol of the ophthalmic examination is required). The long protocol is used in the differential diagnosis of various retinal problems, and also when ophthalmic lesions are absent. It is the method used for the early diagnosis of hereditary retinal disorders as well. The long protocol produces information about the different retinal cell types, while the brief protocol (rapid protocol) reveals the mixed rod-cone responses [20]. The examination is based on the active responses of the photoreceptors stimulated by a light of various intensities [5]. Electroretinography requires very specific conditions during examination in order to avoid adverse factors affecting the results of the examination. All of the factors affecting the wave form recordings of the ERG can be divided into physiological or instrumented-related [17]. The proper interpretation of the results requires collecting a data base in which all of the physiological and instrumental aspects have been taken into consideration [20].

RETINA

The retina is the organ transducing light into neuronal signals that are perceived as a visual image. The retina is divided into ten layers or can be subdivided into the neuroretina and the retinal pigment epithelium which is responsible for the nutrition of the outer layers. The neuroretina, with several cell layers, consists of the photoreceptors in one of the outer layers and is responsible for vision [15, 22].

At the moment of birth, until 6-9 week of age, the retina consists of an immature- neurosensory layer which is separated from a pigmented epithelium. The immaturity of the retina is specific for different species and can be noticed in the histopathological examinations or by electroretinography [15].

Rods and cones are the photoreceptors responsible for vision. The number of photoreceptors and their type varies depending upon the different species and the specifics concerning the nature of their external environment and ecology. Humans, as with dogs and cats, have a rod-cone type of retina. However, cats have a much higher concentration of rods than humans. This difference means that cats have

superior night vision, but their visual acuity is relatively limited [24]. Rods are sensitive to low levels of light and to small changes of illumination. Rods are responsible for scotopic vision – dim environments. Rods have low visual resolution in various grey tones. They are useful in the detection of movement. Cones are responsible for photopic vision, in high level of illumination — daylight. They determine high-resolution vision that is important while observing the details. Cones contain pigments for color vision which makes them responsible for general color vision and they are different in different animals according to their cone types [15, 22].

In photoreceptors containing photopigments, after exposure to light, chemical energy is produced which is converted into electrical energy, which is transmitted by the optic nerve, via the optic chiasm, optic tracts, lateral geniculate body, and optic radiations, to the visual cortex [22].

During an ERG examination, the retina is illuminated with various intensities of light. Light induces electrical charges in the cells of the retina which cause an electrical response. The response is the summation of the electrical potentials that result from light induced changes in the movement of sodium and potassium ions within the extracellular space. This response, as wave forms in the electroretinograms, are measured and analyze during the ERG examination [20].

ELECTRORETINOGRAPHY

Electroretinography is a non-invasive diagnostic method of measuring the electrical response of retinal photoreceptor cells [16]. This specific response is caused by the standardized selective stimulation of individual retinal cells types. Every few years, updated guidelines for ERG examination are published which define the conditions for the reproducible recordings based on the anatomy and physiology of a patient and allow for the possibility of comparing results worldwide [23]. According to the guidelines for the clinical electroretinography in the dog updated in 2012, the full examination should consist of four stages.

The recommended protocol:

1. Dark adapt for 20 min while evaluating the rod function and the dynamic process of dark adaptation every 4 min (for practical reasons, the first flash (at 0 min) may be

delivered after 10 s of dark adaptation and the subsequent flashes after 4, 8, 12, 16 and 20 min); with flash intensity preferably 0.01 cd s.m^{-2} or 0.02 cd s.m^{-2} . Optional: perform the dark-adapted intensity/ response series; with flash intensity increasing in at least 7 steps from below b-wave threshold ($B0.001$ or $B0.002 \text{ cd s.m}^{-2}$) to $C3 \text{ cd s.m}^{-2}$.

2. Test the mixed rod–cone response to a 3.0 cd s.m^{-2} flash in the dark. Optional: perform the dark-adapted, high-intensity flash test; with flash intensity 10 cd s.m^{-2} .

3. Test the cone function after 10 min of light adaptation (background light at 30 cd s.m^{-2}); with flash intensity of 3 cd s.m^{-2} .

4. Perform the 30 Hz flicker test at 3 cd s.m^{-2} with a rod suppressing background light of 30 cd s.m^{-2} [6].

The following protocol guarantees the separate examination of rods and cones. According to the knowledge of the photoreceptor's physiology and biochemistry, in order to examine the cones in light adaptation, a high flicker rate and high intensity of stimulation is required. In order to examine the rods in dim stimulation, a low flicker rate and dark adaptation at the beginning are needed. To illustrate the rod's sensitivity for dim light in comparison to the required energy of cones, rods are activated by 1–5 photons, while cones need 1,000 photons [23]. Various intensities of light cause various nerve cell reactions in the retina. Light intensity should be determined according to the transparency of the cornea and the lens.

According to the recording electrode type and the distance of the reference electrode from the eye, differences have been noticed in the ERG of the dog that may have influence on the interpretation of the ERG results and assessment of the retinal function evaluation. The results obtained using these different types of electrodes cannot be directly compared [17]. Four electrodes are used to perform the ERG examination. One corneal lens electrode is placed on the cornea; two reference electrodes are placed in the temporal cantus of the eye; and a grounded electrode is positioned on the top of the skull [6, 17]. The space between the corneal electrode and the cornea should be filled with a solution. The ionic solution used in the ERG has to be nonirritating and with a viscosity of less than 0.5% of a methylcellulose solution [6]. The retina has to be illuminated evenly; the position of the pupils and its dilatation has to be equal in both eyes [6, 20]. After retinal stimulation, the electroretinogram should be received as curves with all its component: a, b, c, and d waves.

The a-wave consists of the first negative deflection from the photoreceptors. It corresponds to the hyperpolarization of the photoreceptors after the light stimuli [7].

The b wave is the first positive deflection of the depolarization from non-neuronal glia cells in the inner nuclear layer [7]. It is known that the b-wave is associated with Muller cells and bipolar ON cells. According to Kofuij et al., the connection between the b wave and the Muller cells has been excluded [12]. On the ascending limb of the b-wave there are small wavelets, i.e. rhythmic waves known as oscillatory potentials (OPs) [32].

The c-wave is a slow positive, deflection from the pigmented epithelium and the Muller cells hyperpolarization [6, 7].

The d-wave is a positive peak and a late off type response [33].

The i-wave is the following positive peak [14].

Every retinal disorder determined by the results of the examination can be interpreted from the electroretinogram by the analysis of the implicit time and amplitude of the waves. The amplitude of the a-wave is measured from the pre-stimulus baseline to the trough of the a-wave, and the amplitude of the b-wave is measured from the trough of the a-wave to the peak of the b-wave. The implicit time of the a-wave is measured from the onset of the stimulus to the trough of the a-wave and the implicit time of the b-wave is measured from the onset of the stimulus to the peak of the b-wave [17].

FACTORS AFFECTING THE ELECTRORETINOGRAM

The ERG examination requires specific conditions due to various factors having an impact on the recording. Not only anatomical and physiological differences between species have influence on the ERG, but also noise and surrounding light are factors [21]. Differences between breeds [6, 1] and age groups [26, 30] cause differences in recordings like a decrease of the amplitude in older patients [27]. All parameters like the patient's body temperature $38\text{--}39^\circ\text{C}$ and oxygenation should be kept stable to avoid affecting factors [6, 27]. The preparation of the patient and technical aspects of examination, like the placement or type of electrode used are very important. To prevent pre-exposure of light from instruments during pre-surgical ophthalmic

examinations, the ERG patient should spend minimally 60 minutes in a normally illuminated room [6].

The ERG examination needs to be performed in a dark dimly lit room with red light that allows for the simplified monitoring of the patient during the examination. In veterinary ophthalmic electroretinography examinations, the patient needs to be placed under general anesthesia to exclude blinking, movement of globe and other disturbances that may have an adverse influence on the recordings. The position and size of the pupils must be proper to ensure equal exposure to the light on the retina. It is necessary to use a lid speculum to retract the eyelids and stay sutures to position eye globe [6, 17, 28]. A stable depth of anesthesia is necessary during the examination [6]. To avoid all affecting factors, the anesthetic protocol has to be taken into consideration. According to the collected database for anesthetics, diazepam reduces the a-wave amplitude, while barbiturates decrease it in high doses but also increase it in low doses [23]. It has been demonstrated that a xylazine and ketamine combination has not only low impact on implicit time and amplitudes but also do not cause eye globe rotation and miosis [11, 13].

CLINICAL ASPECT

The electroretinography examination is useful even during an ophthalmic examination of the fundus when the optic system is not transparent — in cases of corneal edema, hyphaema, hypopion, or vitreous hemorrhage [20].

The ERG is a basic examination before cataract surgery, as it determines the eventual therapeutic strategy. Cataracts often occurs in geriatric patients along with other ocular abnormalities. The revealed abnormalities in pre-surgical evaluation of the retina excludes patient from phacoemulsification [8, 16, 20, 22].

In dogs, some of the retinal disorders are congenital and often not diagnosed until the onset of severe visual problem in older patients. It is recommended to perform an ERG examination prior to breeding in order to prevent genetic defects. The ERG gives the possibility of an early diagnosis of hereditary eye diseases that is significant in selective breeding [16, 20, 22, 27]. The analysis of the electroretinogram of patients with progressive developing diseases like PRA gives knowledge about the pathogenesis of retinal disorders and its stages which is important in veterinary ophthalmology [20]. It has been found in Abyssinian crossbred

cats that progressive retinal degeneration in heterozygous carrier cats could be differentiated from homozygous affected cats prior to clinically evident retinal degeneration by comparing the ERG b-wave to a-wave ratio [10]. Using ERG examination we can differentiate vision defects caused by retinal damage from optic nerve disorder like optic neuritis. Blindness in an early stage of SARD typically is not accompanied by any ocular findings, especially in the retinal layers [18]. In the absence of funduscopic abnormalities, ERG is required to detect the disease [16, 20]. Glaucomatous optic neuropathy causes potentially detectable progressive loss of retinal ganglion cells; electroretinography may be useful in the diagnosis and evaluation of glaucoma [2].

In some cases, like PRCD in English Cocker Spaniels, and Labradors, or CRD in short haired Dachshund, changes in the ERG can be found even 3 years before clinically observed fundic changes appear. This examination over reaches real symptoms of retinal diseases and gives the possibility of treatment. In human medicine, but also veterinary medicine, thanks to ERG, progress in gene therapy helps to prevent development of diseases, for example in the case of successful gene therapy in older Rpe65-deficient dog [19].

ERG/MRI/ COLORIMETRIC PRL DEVICE

In veterinary ophthalmology there are many possibilities (colorimetric PRL Device, ERG, basic ophthalmoscope examination or even MRI) for detecting the function of the retina or loss of vision. Some of these methods require sedation of the patients or general anesthesia. All of these methods requires specialist equipment, specialist conditions and knowledge for the interpretation of the results. According to research published in 2013, colorimetric PLR may be a useful method for determining whether electroretinography (ERG) or magnetic resonance imaging (MRI) should be performed on dogs with acute blindness. Electroretinography is the final examination that needs sedation and special conditions, but in objective ways, provides detailed information about photoreceptor function [31].

CONCLUSIONS

In order to perform ERG examination properly, it is necessary to consider all physiological and instrumental

affecting factors. It is a basic pre-surgical examination before cataract surgery. It is used worldwide in the differential diagnosis of retinal disorders, also for the early hereditary retinal diseases with absences of ophthalmic lesions. The standardized selective stimulation of the ERG examination provides information about the function of the retina, its cells, and eventual problems. In addition ERG can determine the separate evaluation of rods and cones function. The ERG examination is based on the electrical stimulation of the retina that is recorded as curves with specific components. The ERG curves are filtered, averaged and graphically represents the response of the retina. The retinal disorders determined are the results of the examination of the data interpreted from the electroretinogram by the analysis of the implicit time and amplitude of the waves. Every few years, updated guidelines for ERG examinations are published to standardize the examination all over the world. The last guidelines for dog's electroretinography with full standardized protocol were published in 2012.

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